

Urinary excretion of heat-shock protein 70 in patients with familial mediterranean fever

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Abstract

Aim: In this study, we aimed to determine the urinary excretion of HSP70 in patients with familial Mediterranean fever (FMF).

Material and Methods: Children with FMF attack-free period and FMF attack and healthy controls were enrolled in the study. Characteristics of patients and laboratory results were recorded. Urine HSP70 levels, urine creatinine were studied, and urinary excretion of HSP70 was calculated.

Results: Mean levels of urine HSP70 in patients with FMF attack-free period were significantly lower than that of controls ($p = 0.01$). The mean urinary excretion of HSP70 was higher in patients with an FMF attack than that of patients with FMF attack free period, but similar with controls. The mean HSP70 and urinary excretion of HSP70 were not different in children with homozygous MEFV mutation, in children with M694V mutation and others ($p > 0.05$). There was negative correlation between urinary excretion of HSP70 and the age of patients and controls ($r = -0.284$, $p < 0.01$). There was no statistically significant correlation between urinary excretion of HSP70 and leukocyte counts, CRP, ESR, fibrinogen levels ($p > 0.05$).

Discussion: Urinary excretion of HSP70 increased in FMF patients during the attack period as it is a stress factor. Lower levels of urinary excretion of HSP70 in FMF patients with attack-free period may be related to the anti-inflammatory activity of colchicine. Urinary excretion of HSP70 was negatively correlated with the age of pediatric patients. MEFV mutation type as homozygous mutation and M694V mutation did not affect levels of urinary excretion of HSP70.

Keywords

Familial Mediterranean Fever, Heat Shock Protein, Colchicines, FMF Attack, MEFV Mutation

DOI: 10.4328/ACAM.20909 Received: 2021-10-19 Accepted: 2021-11-04 Published Online: 2021-11-14 Printed: 2022-02-01 Ann Clin Anal Med 2022;13(2):185-188

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Introduction

Heat-shock proteins (HSPs) are a group of intracellular proteins and can be markedly upregulated by all cells under conditions of stress as increased temperature (fever); nutritional deficiency; exposure to pro-inflammatory mediators; oxidative stress; treatment with non-steroidal anti-inflammatory drugs and viral infection [1-4]. HSPs that are classified into about six families (including the HSP10, HSP40, HSP60, HSP70, HSP90 and HSP100 families) on the basis of their monomeric molecular weight, have crucial housekeeping functions and act as molecular chaperones that are important for the survival of cells [5-6]. HSP70 is predominantly an intracellular protein, which can be released to extracellular milieu as a result of tissue damage or cellular necrosis. Besides, active secretion of HSP70 may occur in trauma, sepsis, autoimmune diseases, and inflammatory conditions [7-10]. Extracellular HSP70 negatively regulates the production of pro-inflammatory cytokines of monocytes and contributes to dampen the inflammatory response [11]. HSP70 production localizes in renal tubular cells in the kidney [12]. Recently, various studies have shown that HSPs, particularly HSP60 and HSP70, constitute a group of ‘autoantigens’ with the potential to trigger immunoregulatory pathways, which can suppress immune responses that occur in human inflammatory diseases, such as rheumatoid arthritis (RA), type 1 diabetes, and possibly atherosclerosis and allergy [5-6]. Therefore, we aim to determine the urinary excretion of HSP70 in patients with FMF.

Material and Methods

The study design was prospective. The study was approved by the local ethics committee (date: 30.05.2017, number:13/07), and informed consent was obtained from all patients and controls. The study was conducted in accordance with the Declaration of Helsinki. Patients with a diagnosis of FMF due to Tel Hashomer criteria were enrolled in the study. The inclusion criteria for the patient group were as follows: <18 years old and receiving only colchicine treatment. The exclusion criteria were age >18 years, any other rheumatological disease, renal disease, and any other medication besides colchicine, active infection, and a history of infection 14 days before urine sampling. Characteristics of patients were recorded with the results of MEFV mutation type, follow-up period, FMF attack at the time of urine sampling, presence of proteinuria, presence of amyloidosis, results of leukocyte (count/mm³), erythrocyte sedimentation rate (ESR, mm/hour), C-reactive protein (CRP, mg/l) and fibrinogen (mg/dl). FMF attack was determined with clinical symptoms as fever of unknown origin, abdominal pain, arthritis or arthralgia, chest pain, dyspnea and any increase of acute-phase reactants. Fever was determined as an axillary fever > 38°C. The control group consisted of healthy children who were admitted to the general pediatric outpatient clinic for routine evaluation. The exclusion criteria were any systemic disease, any medication, active infection, and a history of infection 14 days before urine sampling. Mid-stream urine samples from the patient and control groups were centrifuged at 2000G for 10 minutes and stored at -80 °C. Urine HSP70 level was determined using ELISA kits (ChemWell)

and urine creatine levels were determined using the calorimetric Jaffe method (Roche). Excretion of urine HSP70 was calculated with the ratio of urine HSP70 to urine creatinine levels.

Statistical Analysis

IBM SPSS Statistics 20 program was used for statistical analysis. Mean, standard deviation, median, minimum and maximum values were given in descriptive statistics for continuous data and percentage values were for discrete data. Normality tests were performed using the Shapiro-Wilk test. Differences in proportions and continuous data were tested using the Pearson chi-square test and Mann-Whitney U test, respectively. Correlation analysis was evaluated using Spearman's Correlation Coefficient. P < 0.05 was accepted as statistically significant.

Results

Characteristics of patients and controls

Fifty-five children in the patient group and 46 children in the control group were enrolled in the study. The mean age of the patient and control groups was 12.18 ± 4.06 years and 11.96 ± 4.11years, respectively (p > 0.05). Totally 50.9% (n = 28) of patient group and 47.8% (n = 22) of control group were male, respectively (p > 0.05). The mean duration of follow- up period in the patient group was 5.91 ± 4.01 years (range: 1 – 13 years). Patients were categorized according to the presence of an FMF attack at the time of urine sampling. Group 1 consisted of 44 patients with FMF attack-free period and group 2 consisted of 11 patients with FMF attack. All patients have received only colchicine treatment. The follow-up periods of patients in groups 1 and 2 were similar (respectively 6.25 ± 3.98 years and 4.54 ± 3.95 years, p > 0.05). Group 2 had higher leukocyte, CRP, sedimentation and fibrinogen levels than group 1 (Table 1).

Table 1. Characteristics of patients with FMF attack-free period (group 1) and with FMF attack (group 2)

	Group 1 (n=44)	Group 2 (n=11)	P
Age (years)	12±3	11±5	>0.05
Sex (male/female)	23/21	5/6	>0.05
Follow-up period (years)	6.25±3.98	4.54±3.95	>0.05
Leukocyte (mean± SD, count/mm ³)	7137±1641	8581±2967	<0.05*
CRP (median, mg/l, range)	0.1 (range: 0.0-5)	10 (range: 1-170)	<0.01*
ESR (mean± SD, mm/hour)	5±4	19±12	<0.05*
Fibrinogen (mean± SD, mg/dl)	273±49	379±89	<0.05*

*p<0.05 was significant

Table 2. MEFV mutation analysis of patients (n=50)

Homozygous n=16	Heterozygous n=10	Compound Heterozygous n=20	Triple Heterozygous (n=4)
M694V/M694V (n=10)	E148Q/- (n=3)	M694V/V726A (n=5)	R202Q/M694V/M680I (n=2)
M680I/M680I (n=3)	M694V/- (n=3)	M694V/M680I (n=5)	R202Q/E148Q/M694V (n=2)
R202Q/R202Q (n=3)	R202Q/- (n=3)	M694V/E148Q (n=4)	
	A744S/- (n=1)	M680I/V726A (n=3)	
		M694V/R761H (n=2)	
		E148Q/K695R (n=1)	

Table 3. Urine HSP70 levels and urinary excretion of HSP70 in patients due to mutation type

	Homozygous MEFV mutation (n=16)	Other mutation types (n=39)	Controls (n= 46)	p*
Urine HSP70 (pg/dl)	44.44±22.28	45.20±42.60	56.33±27.67	>0.05
Urine HSP70/creatinine(pg/mg)	57.26 ±47.68	74.92±74.54	77.00±53.48	>0.05

	MEFV mutation with M694V (n= 33)	Other mutation types (n=22)	Controls (n= 46)	p*
UrineHSP70 (pg/dl)	43.22±27.41	49.83±47.61	56.33±27.67	>0.05
Urine HSP70/creatinine(pg/mg)	72.53±62.55	77.10±65.00	77.00±53.48	>0.05

*p<0.05 was significant

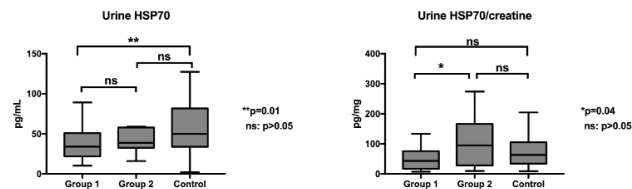


Figure 1. Urine HSP 70 levels and urinary excretion of HSP70 in patients with FMF attack-free period (group1), with FMF attack (group 2) and controls

There were no patients with amyloidosis and proteinuria.

MEFV mutations in patients

Totally, 16 patients (32%) had a homozygous mutation, 10 patients (20%) had a heterozygous mutation, 20 patients (40%) had a compound heterozygous mutation, and 4 patients (5%) had triple MEFV gene mutation. Five patients had no mutation on MEFV gene. The most common MEFV mutation was M694V with 20% (n = 10) homozygous, 6% (n = 3) heterozygous, 32% (n = 16) compound heterozygous and 4% (n = 2) triple heterozygous characteristics (Table 2).

Urinary excretion of HSP70

Mean levels of urine HSP70 in group 1, group 2 and controls were 41.52 ± 27.02 pg/ml, 58.81 ± 65.12 pg/ml and 56.33 ± 27.67 pg/ml, respectively. Mean levels of urine HSP70 in group 1 were significantly lower than that of controls (Figure 1). The mean urinary excretion of HSP70 in group 1, group 2 and controls were 60.35 ± 60.24 pg/mg, 106.62 ± 86.76 pg/mg and 77.00 ± 53.48 pg/mg respectively. The mean urinary excretion of HSP70 was higher in group 2 than that of group 1, but similar with controls (Figure1). The mean HSP70 and urinary excretion of HSP70 was not different in children with a homozygous MEFV mutation, in children with a M694V mutation and others (p > 0.05, Table 3). There was negative correlation between urinary excretion of HSP70 and the age of patients and controls (r = -0.284, p < 0.01). There was no statistically significant correlation between urinary excretion of HSP70 and follow up period of patients, leukocyte counts, CRP, ESR, fibrinogen levels (p > 0.05).

Discussion

In this study, we analyzed urine HSP70 levels and urinary excretion of HSP70 in FMF patients. Interestingly, we demonstrated lower HSP70 levels in FMF patients with attack-free periods than in healthy controls, and higher urinary excretion of HSP70 in FMF patients with attacks than that of patients with attack-free periods. There was no difference in urinary excretion of HSP70 due to MEFV mutation type. Urinary excretion of HSP70

was negatively correlated with the age of patients. There was no correlation between urinary excretion of HSP70 and follow-up period of patients and laboratory parameters.

In addition to their physiological roles, heat shock proteins (HSP) have been presented to play a role in the pathogenesis of various immune-mediated disorders such as infections (tuberculosis and chlamydia), autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis), vascular thrombosis (atherosclerosis), malignant disorders, dermatomyositis, inflammatory muscle disease, organ transplantation and cardiac surgery [5-6]. Serum levels of HSP70 were increased in patients with Behçet's Disease (BD) [13]. In another study, free human HSP70 and anti HSP70 antibodies were both elevated in patients with BD, so the authors concluded that HSP70 mediated innate and adaptive immune responses may participate in pro-inflammatory cytokine activation and tissue destruction in BD [14]. In our study, it was noteworthy that urine HSP70 levels and urinary excretion of HSP70 were lower in FMF patients with attack-free period. Interestingly, urine HSP70 levels and urinary excretion of HSP70 in FMF patients with attacks were similar with healthy controls. FMF patients in our study received only colchicine treatment. Colchicine is one of the oldest medicines derived from the colchium autumnale plant. It inhibits neutrophil migration seen in many inflammatory diseases at the cellular level, and it is a vinca alkaloide connecting to tubulin [15-16]. Lower urine HSP70 levels and urinary excretion of HSP70 in FMF patients with attack-free period may be related to two reasons: one reason may be increase of HSP70 due to inflammation of attack, as it is a stress factor, and the second reason may be anti-inflammatory activity of colchicine in FMF patients with an attack-free period. It is known in the literature that a synthetic derivative of colchicine was found to have HSP70-inhibiting activity [17]. Loss of HSP70 function induced defects in mitotic spindles and resulted in altered mitosis progression and subsequent cell death. HSP70 was also required for the assembly of the mitotic centrosome and bipolar spindles [18]. The causative gene of FMF was first described in 1997 and named the Mediterranean Fever gene (MEFV), which encodes a protein named pyrin. The pyrin protein has functions in apoptotic and inflammatory pathways [19]. Mutations of the pyrin lead to upregulation of the inflammatory cytokines, especially interleukin-1B. Over 100 mutations related to FMF have been identified, and mutations located in exon 10 such as M694V, M694I, and M680I are related to a more severe disease phenotype [19,20]. M694V mutation was the most common mutation type in our study, and urine HSP70 levels and urinary

excretion of HSP70 was similar in patients with homozygous mutations, M694V mutation type and others. Aged organisms have a reduced ability to respond to stimuli or stress and to maintain homeostasis [21]. It was reported that induction of HSP70 expression is significantly reduced in cells from old rats and that the reduced expression is due to a deficit in the transcription of the hsp70 gene. Thus, it appears that changes in the induction of HSP70 transcription are closely associated with the aging process in rats [22]. In the literature, there have been no reports of age-related change in HSP levels in the pediatric age group. But our study demonstrated that urinary excretion of HSP70 was negatively correlated with the age of patients in the pediatric age group. Limitations of our study were a small number of FMF patients with attack period and the lack of comparison of urinary excretion of HSP70 in the same FMF patients during attack and attack-free period. In order to demonstrate the effect of colchicine on urinary excretion of HSP70 more clearly, further studies can be planned before colchicine treatment and while receiving colchicine treatment in the same patient. In conclusion, our study demonstrated that urinary excretion of HSP70 increased in FMF patients during attack period, as it is a stress factor. Lower levels of urine HSP70 levels and urinary excretion of HSP70 in FMF patients with attack-free period are interesting, and the reason for this result may be related to the anti-inflammatory activity of colchicine. Urinary excretion of HSP70 was negatively correlated with the age of pediatric patients. MEFV mutation type, as the homozygous mutation and M694V mutation, did not affect levels of urinary excretion of HSP70.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: None

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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How to cite this article:

Eren Yıldız, Arife Uslu Gökçeoğlu, Nermin Dindar Badem, Bülent Alioğlu. Urinary excretion of heat-shock protein 70 in patients with familial mediterranean fever. *Ann Clin Anal Med* 2022;13(2):185-188